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Fluid resuscitation with lactated Ringer's solution vs normal saline in acute pancreatitis: A triple-blind, randomized, controlled trial

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Abstract

Background: Little is known regarding the optimal type of fluid resuscitation in acute pancreatitis (AP).

Objective: The objective of this article was to compare the effect of lactated Ringer's solution (LR) vs normal saline (NS) in the inflammatory response in AP.

Methods: We conducted a triple-blind, randomized, controlled trial. Patients ≥ 18 admitted with AP were eligible. Patients were randomized to receive LR or NS. Primary outcome variables were number of systemic inflammatory response syndrome (SIRS) criteria at 24 hours, 48 hours and 72 hours and blood C-reactive protein (CRP) levels at 48 hours and 72 hours. In vitro complementary experiments were performed to further explore the interaction between pH, lactate and inflammation.

Results: Nineteen patients receiving LR and 21 receiving NS were analyzed. The median (p25–p75) number of SIRS criteria at 48 hours were 1 (1–2) for NS vs 1 (0–1) for LR, $p = 0.060$. CRP levels (mg/l) were as follows: at 48 hours NS 166 (78–281) vs LR 28 (3–124), $p = 0.037$; at 72 hours NS 217 (59–323) vs LR 25 (3–169), $p = 0.043$. In vitro, LR inhibited the induction of inflammatory phenotype of macrophages and NF-κB activation. This effect was not observed when using Ringer's solution without lactate, suggesting a direct anti-inflammatory effect of lactate.

Conclusions: Lactated Ringer's is associated with an anti-inflammatory effect in patients with acute pancreatitis.

Keywords

Acute pancreatitis, fluid resuscitation, lactated Ringer's solution, randomized controlled trial, systemic inflammatory response syndrome/prevention and control

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Key summary

Summarize the established knowledge on this subject

- Fluid resuscitation is widely considered to be an essential part of the management of acute pancreatitis (AP) but little is known about the optimal type of fluid.
- An open-label, randomized, controlled trial (RCT) suggested that normal saline, when compared to lactated Ringer's solution, is associated with a pro-inflammatory effect in patients with AP.
- This pro-inflammatory effect has been attributed to normal saline-induced hyperchloremic metabolic acidosis.

What are the significant and/or new findings of this study?

- Lactated Ringer's solution was associated with an anti-inflammatory effect in patients with AP in a triple-blind RCT.
- This effect is due to direct anti-inflammatory properties of lactate, and not to metabolic acidosis.

Introduction

Fluid resuscitation is widely considered to be an essential part of the management of acute pancreatitis (AP), but there is a lack of consensus on specific recommendations regarding the type of fluid to use and the optimal rate of fluid administration.[1]

Dr Bechien Wu and colleagues published a four-arm (2×2) factorial design, pilot randomized, controlled trial in 2011.[2] A total of 40 patients were randomized to one of four treatments arms: (1) goal-directed fluid resuscitation with lactated Ringer's solution (LR), (2) goal-directed fluid resuscitation with normal saline (NS), (3) standard resuscitation with LR, or (4) standard resuscitation with NS. There was a significant reduction in systemic inflammatory response syndrome (SIRS) and C-reactive protein (CRP) levels after 24 hours among patients resuscitated with LR compared with NS. The authors suggested that NS is detrimental for AP because of hyperchloremic acidosis. Since the publication of this trial, most guidelines have recommended LR as the fluid of choice for resuscitation in AP.[3,4] However, randomization alone does not preclude the possibility of systematic differences: If patients, clinicians, or assessors are aware of treatment assignments, this may influence reporting or measurement of the outcome and introduce bias.[5,6] Furthermore, the study had four arms of treatment. This prompted us to design a triple-blind, randomized, controlled trial (RCT) comparing LR with NS, with a homogeneous fluid resuscitation protocol to investigate whether patients receiving LR develop a lower inflammatory state than patients receiving NS. Additionally, we evaluated the effect of these fluids on the inflammatory response on macrophages to improve our knowledge about the physiopathology of fluid resuscitation.

Materials and methods

Trial design

The trial was a single-center, triple-blind RCT.

Participants

Patients aged 18 years or older who initially presented to the emergency room and were subsequently admitted in our center with a first episode of AP were eligible for study enrollment.

AP was defined as two of the following three criteria: (1) characteristic abdominal pain, (2) serum amylase and/or lipase greater than three times the upper limit of normal, and (3) cross-sectional abdominal imaging demonstrating changes consistent with AP.[7] Patients included under a false diagnosis of AP (for example, patients with acute cholecystitis) were not analyzed (as they did not have a final diagnosis of AP). The exclusion from analysis of those patients was determined during their hospitalization (therefore, before blinded statistical analysis) by the trial monitor.

The exclusion criteria were: time from pain onset to randomization >24 hours, known history of renal disease (basal creatinine >2 mg/dl, patient under chronic hemodialysis), greater than New York Heart Association class II heart failure, chronic lung disease requiring supplemental home oxygen, active acute infection (including acute cholecystitis and acute cholangitis), hypernatremia (serum sodium>145 mEq/l) or hyponatremia (<135 mEq/l), rhabdomyolysis, metastatic malignant disease, autoimmune diseases associated with inflammation (including inflammatory bowel disease), chronic infection (e.g. human immunodeficiency virus (HIV) and tuberculosis).

Interventions

Patients were randomized to receive LR or NS as fluid resuscitation. All patients received 1000 ml of 10% dextrose solution in addition to the study fluid.

Patients with hematocrit >44% and/or two or more SIRS criteria and/or blood urea nitrogen>20 mg/dl and/or signs of dehydration or hypovolemia received more vigorous resuscitation: 15 ml/kg of the study fluid in 60 minutes immediately after randomization, and then 1.2 ml/kg/hour of the study fluid for three days. All other patients received 10 ml/kg of the study fluid in 60 minutes immediately after randomization, and then 1 ml/kg/hour of the study fluid for three days. In patients with oliguria or hypotension, the attending physician could administer boluses of 500 to 1000 ml of the study fluid in 30 to 60 minutes as needed. In case of fluid overload, the attending physician could decrease the study fluid volume rate and use diuretics as needed.

Aims

Our primary aim was to compare the effect of LR vs NS on the systemic inflammatory response of patients with AP. To obtain more information about the relationship between fluid type and inflammation, we performed in vitro experiments to evaluate the anti-inflammatory effect of these fluids in cell cultures, and to explore the interaction between LR, NS, pH, lactate and inflammation.

Primary outcomes

Primary outcomes were the number of SIRS criteria at 24 hours, 48 hours and 72 hours and levels of CRP at 48 hours and 72 hours (CRP peaks at 72 hours[8]).

Other variables

Bicarbonate levels and pH were measured from venous blood gas at 24 hours, 48 hours and 72 hours from randomization. We analyzed the incidence of local complications (pancreatic necrosis and peripancreatic necrosis, defined by the revised Atlanta classification[7]), systemic complications (persistent organ failure, defined by the revised Atlanta classification[7]), the incidence of moderate-to-severe disease according to the revised Atlanta classification, need for intensive care unit (ICU) admission, nutritional support, invasive treatment, as well as hospital stay and mortality.

Sample size

For sample calculation, we focused on blood CRP levels. In a prospective cohort of our patients[9] who received fluid resuscitation based on NS, mean blood CRP levels was 160 mg/l (standard deviation 111). We aimed to detect a 100-mg/l difference. We estimated that a sample of 40 patients would provide the study with at least 80% power, at a two-sided alpha level of 5%.

Randomization and blinding

The Pharmacy Department of the Alicante General University Hospital (A.G.) conducted the randomization and blinding. A random number list was generated by computer (SPSS 20.0, see below) and used for treatment assignment (simple randomization, allocation ratio 1:1). LR and NS were repacked in 1000 ml plastic bags and identified only with a number of registration (A.G.). Eligible patients were identified by staff physicians from the Emergency Department or by staff physicians and residents from the Department of Gastroenterology. Participants required written informed consent to be included in the study. For blinded statistical analysis, once the study recruitment was finished, the Pharmacy Department provided a list with the number of registry and two labels: A or B. After the statistical analysis was performed, the Pharmacy Department unmasked the treatment groups (A = NS, B = LR). The whole process of blinding was monitored by the Clinical Pharmacology Department.

In vitro experiments

Cell line. For the in vitro studies on the effect of fluid therapy on inflammatory cells, we used the THP-1 cells. This is a human monocytic cell line that can be differentiated into a macrophage-like phenotype by treatment with phorbol esters and has been extensively used to study monocyte/macrophage functions.[10] Cells were cultured in suspension in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were differentiated to macrophages through a first incubation with 100 nM phorbol 12-myristate 13-acetate (PMA) (Sigma Aldrich, St Louis, MO) for 48 hours. After that, the PMA-containing media was discarded and replaced with fresh media without PMA for a further 24 hours. Cells were grown in a humidified atmosphere of 95% air, 5% CO_2 at 37°C. The experiments were repeated four times.

pH. To analyze the effect of fluids on pH, increasing volumes of NS or LR were added to culture media and the resulting pH was measured with a pH-meter (Sartorius, Alcobendas, Spain).

Macrophage activation. To evaluate the activation of macrophages, the RNA expression of interleukin

(IL)-1β was evaluated by reverse transcription-polymerase chain reaction (RT-PCR) and quantitative polymerase chain reaction (qPCR). Cells were challenged with interferon gamma (IFNγ) (100 ng/ml) +lipopolysaccharide (LPS) (100 ng/ml) and treated with NS, LR or, in additional experiments, Ringer's solution without lactate.

Three hours later, total RNA from cells was extracted using the TRizol® reagent (Invitrogen, Carlsbad, CA). RNA was quantified by measuring the absorbance at 260 nm and 280 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Complementary DNA (cDNA) was synthesized from 1 µg RNA sample using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA).

Subsequent qPCR was performed in a DNA Engine, Peltier Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) using iTaq™ Universal SYBR® Green Super mix and the corresponding primers: GAPDH forward: 5'-CTGTGTCTTTCCGCTGTTTTC-3' and reverse: 5'-TGTGCTGTGCTTATGGTCTCA-3'; IL-1β forward: 5'-AAAAATGCCTCGTGCTGTCT-3' and reverse: 5'-TCGTTGCTTGTCTCTCCTTG-3'; tumor necrosis factor alpha (TNFα) forward: 5'-AAC TCCCAGAAAAGCAAGCA-3' and reverse: 5'-CGA GCAGGAATGAGAAGAGG-3'; mannose receptor, C type 1 (MRC1) forward: 5'-GGATGGATGGCT CTGGTG-3' and reverse: 5'-TCTGGTAGGAAACG CTGGT-3'. Reactions were performed in duplicate and threshold cycle values were normalized to GAPDH gene expression. The specificity of the products was determined via a melting curve analysis. The ratio of the relative expression of IL-1β to GAPDH was calculated by using the ΔC(t) formula.

Immunofluorescence. To monitor nuclear factor (NF)-κB translocation, cells were incubated in coverslips and fixed with 3.5% formaldehyde for five minutes at room temperature. The cells were stained with anti-p65 antibody and Alexa fluor 594-conjugated anti-goat secondary antibody. Nuclear localization was examined by fluorescence microscopy.

Statistical methods

Data were analyzed on an intention-to-treat basis. Continuous data were evaluated for normality by the Shapiro-Wilk test and were summarized using mean and standard deviation (SD) or median and 25th percentile–75th percentile (interquartile range) depending on the variable distribution. Differences between the two groups with continuous data were assessed using student-*t* test for normal and Mann-Whitney *U* test for non-normal distributions. Qualitative data were described using percentages and compared by the chi-square, using Fisher test when needed. A two-sided *p* value of less than 0.05 was considered statistically significant. All statistical calculations were performed with SPSS 20.0 (Armonk, NY, USA).

Institutional review board approval

The institutional review board of our center approved the study on December 21, 2011, and the Spanish Drug Agency (Agencia Española de Medicamentos y Productos Sanitarios) approved the study protocol on August 8, 2012.

We followed the CONSORT guidelines for reporting results of the RCT.

Results

Patient enrollment, allocation, follow-up and analysis

From February 2013 to March 2015, 134 patients were assessed for eligibility. Eighty-six did not meet inclusion criteria or met exclusion criteria, and five patients declined to participate. Forty-three patients were randomized, but three of them were later excluded from the study because of an incorrect diagnosis of AP: two of them had an acute cholecystitis on abdominal imaging and one had an acute cholangitis with liver abscesses. Ultimately, 19 patients received LR and 21 NS. All 40 patients received their study fluid and there was no loss to follow-up. Participant flow is displayed in Figure 1.

Baseline characteristics. Baseline characteristics are shown in Table 1.

Fluid administration. Median (p25–p75) study fluid volume administration (ml) was as follows; from 0 to 24 hours after randomization, NS 2400 (2180–3500) vs LR 2160 (1704–3200), $p = 0.313$; from 24 hours to 48 hours, NS 2448 (2160–2900) vs LR 1940 (1572–2250), $p = 0.044$; from 48 hours to 72 hours, NS 2180 (1700–2800) vs LR 1584 (1440–2194), $p = 0.036$. Total 0–72 hours study volume was 6904 (6400–8600) ml for NS and 5900 (4930–7002) ml for LR, $p = 0.045$.

Three patients (14.3%) assigned to NS had signs or symptoms of fluid overload vs 6 (31.6%) assigned to LR, $p = 0.191$.

Primary aim: Effect of study fluid on inflammation. The number of SIRS criteria as well as the proportion of patients with ≥2 SIRS criteria at randomization, and 24 hours, 48 hours and 72 hours thereafter according to arm of treatment are displayed in Table 2. LR showed a

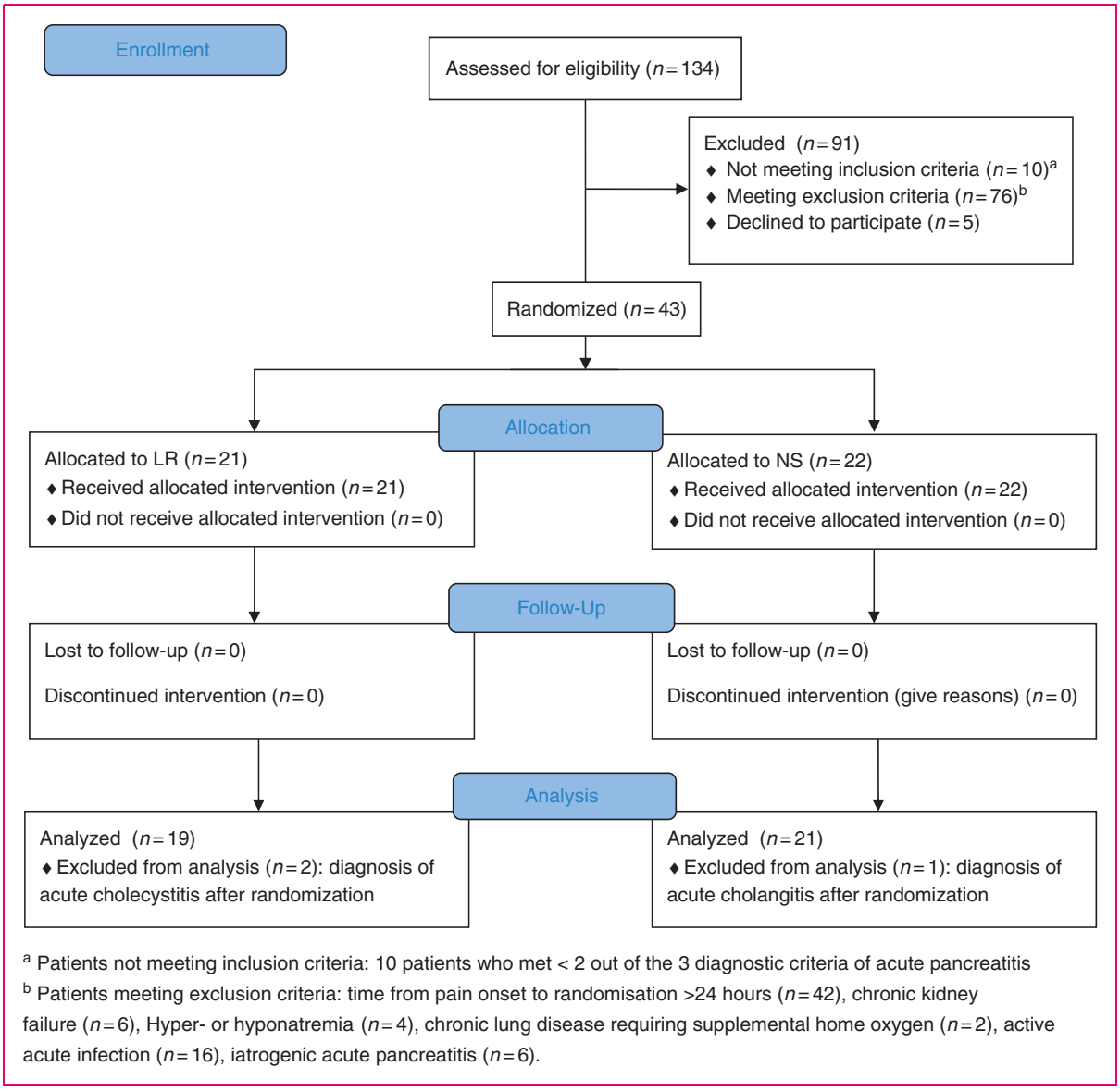

Figure 1. Participant flowchart.
LR: lactated Ringer's; NS: normal saline.

non-statistically significant trend for a lower number of SIRS criteria at 48 hours and 72 hours, $p = 0.060$ and 0.064, respectively. There was a non-significant trend toward a lower frequency of having ≥2 SIRS criteria at 48 hours in the LR group (15.8 vs 42.9% in the NS group, $p = 0.062$). Patients receiving NS had a median (p25–p75) days with ≥2 SIRS criteria during the first week of hospital admission (excluding basal SIRS) of 0 (0–2) days vs 0 (0–1) days in the LR group, $p = 0.124$. Median (p25–p75) CRP levels (mg/l) were as follows; at 48 hours NS 166 (78–281) and LR 28 (3–124), $p = 0.037$; at 72 hours NS 217 (59–323) vs LR 25 (3–169), $p = 0.043$ (Figure 2).

Other outcome variables. Other outcome variables are summarized in Table 3. No significant differences were detected regarding the incidence of pancreatic necrosis, persistent organ failure or mortality; need for nutritional support, invasive treatment, or ICU admission; and length of hospital stay. While there were no significant differences regarding blood pH between both arm treatments, blood bicarbonate levels were higher at 24 hours, 48 hours and 72 hours from randomization in the group receiving LR (Supplementary Table 1). Moderate-to-severe cases received a higher first 72-hour study fluid volume than mild cases: 6900 (6460–9200) ml vs 6100 (4800–6500) ml ($p < 0.004$).

Table 1. Baseline characteristics.

	Normal saline $n = 21$	Lactated Ringer's solution $n = 19$	p
Age (years) Mean (SD)	61.4 (15.5)	63.8 (19.1)	0.661
Male gender n (%)	11 (52.4%)	8 (42.1%)	0.516
Body mass index (kg/m^2) Median (p25–p75)	27.7 (25.1–31.1)	25.2 (22.9–28.3)	0.092
Etiology n (%)			
Gallstones	15 (71.4%)	14 (73.7%)	0.132
Alcohol	4 (19%)	0	
Idiopathic	2 (9.5%)	4 (21.1%)	
Other	0	1 (5.3%)	
C-reactive protein (mg/l) median (p25–p75)	4.8 (1.2–12.5)	3.4 (1.2–7.2)	0.663
SIRS criteria median (p25–p75)	2 (1–2)	1 (0–2)	0.152
SIRS $\geq$2 criteria n (%)	14 (66.7%)	9 (47.4%)	0.218

SIRS: systemic inflammatory response syndrome.

Table 2. Systemic inflammatory response syndrome criteria according to study fluid.

Time from randomization	Variable	Normal Saline	Lactated Ringer's solution	p
Basal	SIRSc	14 (66.7%)	9 (47.4%)	0.218
	SIRSn	2 (1–2)	1 (1–2)	0.181
24 hours	SIRSc	4 (19%)	4 (21.1%)	0.874
	SIRSn	1 (1–1)	0 (0–1)	0.147
48 hours	SIRSc	9 (42.9%)	3 (15.8%)	0.062
	SIRSn	1 (1–2)	1 (0–1)	0.060
72 hours	SIRSc	7 (33.3%)	3 (15.8%)	0.281
	SIRSn	1 (1–2)	0 (0–1)	0.064

SIRS: systemic inflammatory response syndrome. SIRSc: proportion of patients with $\geq$ 2 SIRS criteria, n (%). SRISn: number of SIRS criteria, median (interquartile range).

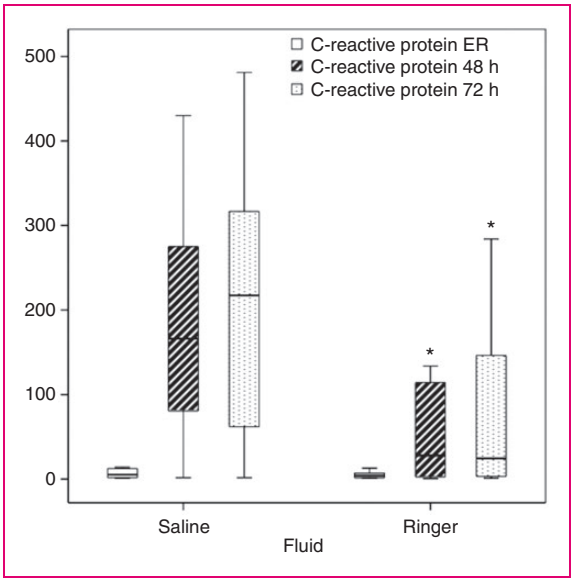

Figure 2. C-reactive protein (CRP, mg/l) at randomization (emergency room, ER), 48 hours and 72 hours. *$p < 0.05$ between patients receiving normal saline (saline) or lactated Ringer's solution (Ringer).

In vitro experiments. In vitro studies revealed that neither NS nor LR has a relevant effect on the pH in cell cultures (Figure 3). The addition of these solutions to culture media resulted in a lower buffer capacity of the cell culture medium due to the dilution, but no differences were found between these two fluids.

By contrast, significant differences were found in the response of macrophages to inflammatory stimulus. Treatment with inflammatory stimulus induces the acquisition of an inflammatory phenotype characterized by expression of IL1β, and TNFα as well as inhibition of MRC1. These changes were significantly inhibited in the presence of 40% LR, while the same volume of NS had no effect on macrophage phenotype (Figure 4). This effect was linked to the presence of lactate since Ringer's solution without lactate lost the anti-inflammatory effect (Figure 4).

Immunofluorescence analysis of p65 subunit of transcription factor NF-κB confirms that the activation induced by LPS + IFNγ is prevented by LR, while neither NS nor Ringer's solution without lactate prevented this activation (Supplementary Figure 1).

Table 3. Other outcome variables.

Outcome	Normal saline $n = 21$	Lactated Ringer's solution $n = 19$	p
(Peri)pancreatic necrosis[a] *n* (%)	10 (71.4%)	4 (40%)	0.211
Need for nutritional support *n* (%)	2 (9.5%)	0	0.488
Need for invasive treatment *n* (%)	1 (4.8%)	1 (5.3%)	1
Persistent organ failure *n* (%)	1 (4.8%)	0	1
ICU admission *n* (%)	1 (4.8%)	0	1
Hospital stay (days) Median (p25–p75)	9 (5–16)	9 (5.8–13)	0.625
Mortality *n* (%)	1 (4.8%)	0	1
Moderate-to-severe AP *n* (%)	10 (47.6%)	7 (36.8%)	0.491

(Peri)pancreatic necrosis: peripancreatic fat necrosis and/or pancreatic gland necrosis. ICU: intensive care unit; AP: acute pancreatitis.
[a]Only patients who underwent contrast-enhanced computed tomography scan (NS: 14, LR: 10).

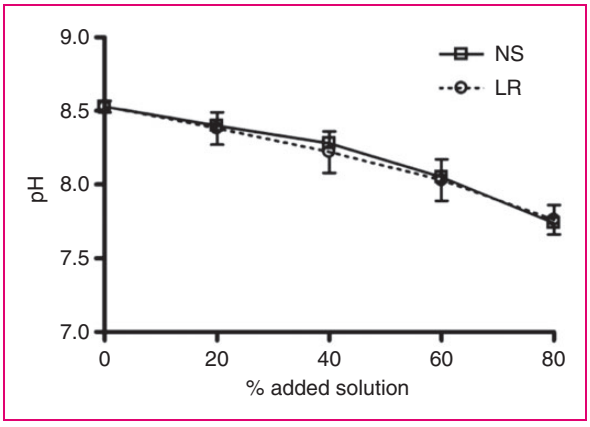

Figure 3. Change in pH after addition of normal saline (NS) or lactated Ringer's solution (LR) to cell culture medium.
Addition of NS or LR to Roswell Park Memorial Institute (RPMI)-1640 medium dilutes the buffering capability and results in a decreased pH. However, no differences were observed between the two solutions.
Values are expressed as mean ± standard error of the mean for three different experiments.

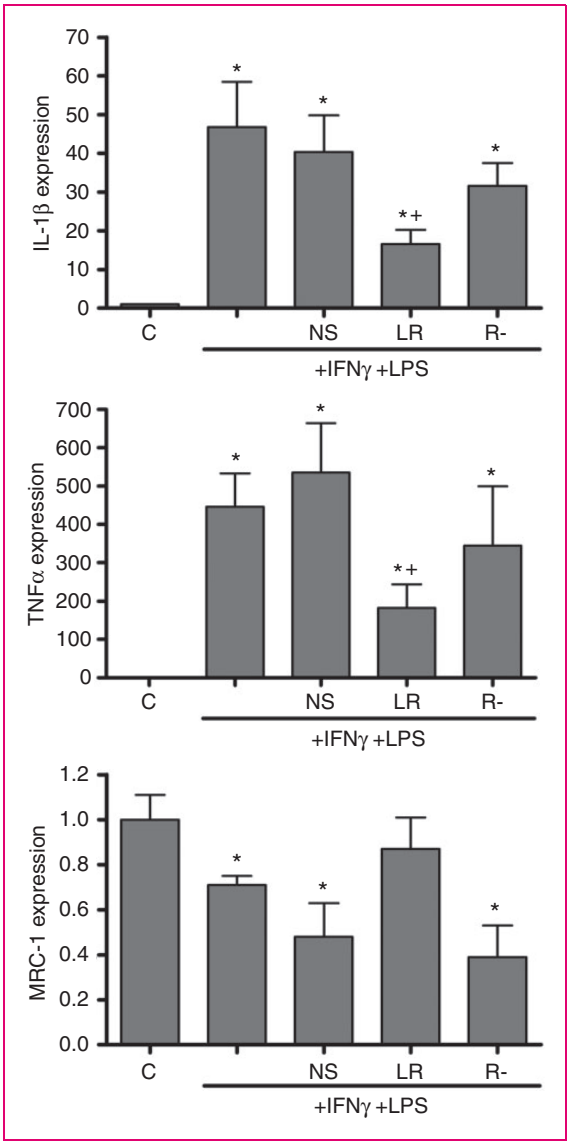

Figure 4. RNA expression of interleukin (IL)-1β, tumor necrosis factor alpha (TNFα) and MRC1 in macrophages in control, normal saline, lactated Ringer's solution (LR) and Ringer's solution without lactate. Values are expressed as mean ± standard error of the mean for four different experiments. *$p < 0.05$ compared with control. +$p < 0.05$ compared to IFN-gamma + LPS without crystalloids.
RNA expression of IL-1β and TNFα in macrophages was significantly increased and mannose receptor, C type 1 (MRC1) was inhibited by treatment with interferon gamma (IFNγ) + lipopolysaccharide (LPS) (100 ng/ml) compared to basal control (C). The presence of 40% normal saline (NS) does not modify these changes while LR significantly reduces the expression of IL-1β and TNFα

Discussion

The present study is the first blinded RCT comparing two different types of fluid for resuscitation in AP. We show that LR, when compared blindly to NS, is associated with an anti-inflammatory effect in AP.

NS is the most commonly used fluid worldwide.[11] Large quantities of this unbalanced/chloride-rich fluid have been associated with hyperchloremic metabolic

acidosis,[12–15] which has been linked to acute kidney injury[16] and immune dysfunction.[17] NS has also been associated with increased morbidity and mortality in septic[18] and critical patients[19] and with increased morbidity in surgical patients[20] when compared to balanced/chloride-restricted crystalloids. These balanced solutions, which include LR, are more similar to extracellular fluid than NS.

Two main mechanisms may explain our findings: an indirect effect related to NS-associated metabolic acidosis or a direct anti-inflammatory effect of LR. Acidosis has been associated with inflammation and necrosis in experimental AP.[21,22] Extracellular acidosis is a danger signal that triggers secretion of inflammatory cytokines like IL-1β in immune cells.[23] Resuscitation with LR is associated with a lower probability of metabolic acidosis, since lactate is metabolized in the liver to bicarbonate. Since local acidification occurs during acute inflammatory processes as AP, it could be hypothesized that LR helps to prevent the drop in pH, thus reducing the activation of inflammatory cells and lowering the intensity of inflammatory response. However, in our study, in vivo and in vitro analysis did not show significant differences in the pH both in human blood samples and in cell cultures. LR was associated with increased blood bicarbonate levels but both treatment arms demonstrated bicarbonate levels within normal limits. This indicates that, at the amounts of fluid administered, there was no significant acidosis in either LR or NS.

By contrast, the presence of LR, but not NS, resulted in significantly reduced activation of macrophages when cultured in the presence of IFNγ + LPS. It prevented the switch to the inflammatory phenotype, characterized by the induction of inflammatory cytokines and inhibition of MRC1. Accordingly, it also inhibits the activation of NF-κB, the main transcription factor involved in inflammatory processes. This inhibition is related to the effect of lactate since the addition of Ringer's solution without lactate to cell cultures resulted in a loss of this inhibitory effect. It is known that short-chain fatty acids as butyrate, propionate and lactate down-regulate the Toll-like receptor (TLR)-induced inflammatory response and promote the alternative anti-inflammatory polarization of macrophages.[24,25] The effect of lactate in suppressing innate immunity has also been observed in experimental models of pancreatitis.[26] Consequently, it could be argued that the more robust anti-inflammatory response observed when using LR is probably related to the inhibitory effect of lactate on the activation of macrophages. Patients resuscitated with LR received lower fluid volume; this finding may be explained by a decreased level of fluid sequestration in patients with a lower inflammatory response. We described previously that SIRS is associated with increased level of fluid sequestration.[27]

The strengths of the study are the following: The type of fluid was given randomly and blindly and fluid resuscitation volume administration was given following a homogeneous protocol; inflammatory markers (SIRS criteria and CRP levels) were followed for 72 hours; and all consecutive patients were enrolled early in the course of disease (in the emergency room, within 24 hours of symptom onset) in contrast to other retrospective studies[28] and RCTs investigating fluid resuscitation in AP[29]; and finally, our results are congruent with the previous open-label RCT by Bechien Wu and colleagues[2] as well as one RCT that showed that aggressive hydration with LR was associated with significantly lower incidence of post-endoscopic retrograde cholangiopancreatography pancreatitis.[30]

The main limitation of our study is that it is focused on inflammation, which is a surrogate marker of important clinical endpoint variables in AP. It is important to note that multiple studies have shown that CRP is a good surrogate marker for severe AP.[31–34] Our study and the previous one[2] should be the basis for a multicenter RCT aiming for more robust outcomes. The number of patients needed for such a study would be large, for example, according to our results, to see differences in the proportion of moderate-to-severe disease (two-sided alpha risk 0.05, beta risk 0.2), 327 patients would be needed per arm of treatment (thus, 654 patients).

Conclusions

LR is associated with decreased inflammation in patients with AP. This effect seems to be related to the inhibitory action of lactate on the inflammatory cells. Given the current general concerns about resuscitation with NS, as well as the congruent results of two RCTs in AP, LR should be considered as the fluid of choice for resuscitation.

Acknowledgments

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Declaration of conflicting interests

None declared.

Ethics approval

This trial is registered under EudraCT Number 2011-004988-68. URL (EU Clinical Trials Register): https://www.clinicaltrialsregister.eu/ctr-search/trial/2011-004988-68/ES.

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Informed consent

All participants in the study were voluntary and expressed written informed consent prior to their inclusion.

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